Insect membrane protein extraction kit

Item No. : EX2020

Specification: 50T/100T

Validity: 2-8℃ storage, valid for one year.

Product content:

Name	50T	100T	Storage conditions
Insect membrane protein extraction reagent A	25mL	50mL	Store at 2-8℃
Insect membrane protein extraction reagent B	500μL	1000μL	Store at 2-8℃
Insect membrane protein solution C	10mL	20mL	Store at 2-8℃
Protease inhibitors	100μL	200μL	Store at -20℃

Note:

1. Protease inhibitors can also be stored at 2-8℃ before use without open lid. Store at -20℃ after opening the lid for use.
2. The protease inhibitor is solid at 2-8℃. Take it out of the refrigerator and return to room temperature or 37℃ water bath for a short time. When it becomes liquid, centrifuge it to the bottom of the tube and then open the lid.
3. Please use the reagent as soon as possible after unpacking!

Product Introduction:

Insect membrane protein extraction kit is an efficient and high-yield membrane protein extraction kit. This kit offers a complete set of reagents for the gentle, efficient extraction of membrane/transmembrane proteins and membrane protein complexes from insect tissues or cultured cells. Proteins extracted and enriched with this kit range from very small molecular weights to very large proteins, from a single transmembrane to more than 7 times, and even some large protein complexes. The extraction process is simple and convenient, and can be completed within 1 hour. The extracted membrane proteins are not only pure, maintain natural activity, and have little cross-contamination.

The protein extracted from this kit can be used for downstream protein research experiments such as Western Blotting, protein electrophoresis, immunoprecipitation, ELISA, transcriptional activity analysis, Gel shift gel blocking assay, enzyme activity determination, etc. The proteins extracted by this kit are active proteins with natural protein conformation.

This kit does not contain EDTA and is compatible with downstream applications such as metal chelation and chromatography.

Bring your own reagents and instruments:

Centrifuge, oscillator, vortex mixer, pipette, refrigerator, ice box, PBS buffer, protein quantification kit, centrifuge tube, suction tip, disposable gloves

Product Features:

1. Easy to use, protein extraction from insect cells and tissues does not need to undergo repeated freeze-thaw, ultrasonic crushing and other pre-treatment.

2. Shorten the time of protein extraction to 30 minutes to 1 hour.

3. Containing protein stabilizer, the extracted protein is stable.

4. The background interference is low when the protein concentration is detected by UV.

5. Protease inhibitor inhibited protein degradation, and the formula of protease inhibitor was optimized. The protease inhibitor mixture consists of 7 separate protease inhibitors; Each inhibitor specifically inhibits one or several protease activities. The composition of the mixture is optimized so that it can inhibit almost all important protease activities, including serine protease, cysteine protease, aspartate protease, alanyl-aminopeptidase, etc.

How to use:

First, use precautions:

1. Please centrifuge the reagent in the rotating cap centrifuge tube briefly before opening the cap, and throw the liquid on the inner wall of the cap to the bottom of the tube to avoid liquid spilling when opening the cap.
2. All reagents must be pre-cooled during the experiment; All utensils must be pre-cooled in a -20℃ refrigerator. The sample must be kept at a low temperature during the whole process.
3. If the solution of protease inhibitor is precipitated during storage, it will not affect the use and should be used normally after dissolution.
4. You can add other protease inhibitor products according to your own experimental needs.
5. extract solution A must be kept at 2-8℃ before use, otherwise it will not be easy to delaminate when downstream membrane protein is extracted.
6. Boiling should be avoided in the loading buffer during membrane protein electrophoresis.
7. The SDS content of loading buffer can be increased during membrane protein electrophoresis.

2. Operation steps

1. Extraction liquid preparation:

Every 500μL extract A, add 2μL protease inhibitor mixture, mix well and put on ice for use.

2. Cut 50mg insect tissue sample with surgical scissors as much as possible. After adding 500μL extract A, fully homogenize with a homogenizer/homogenizer.

3. Transfer the homogenate into a pre-cooled clean centrifuge tube and oscillate at 2-8℃ for 1 hour.

4. Centrifuge the extract at a low temperature of 2-8 C at 12000×g for 5 minutes and remove the supernatant.

5. Add 10μL of extract solution B to the supernatant and mix thoroughly.

6. Bathe in water at 37℃ for 10 minutes.

7 Centrifuge at 1000×g at 37℃ for 3 minutes.

8. At this point, the solution will be divided into 2 layers. Carefully remove the upper layer, leaving

about 50μL of liquid at the bottom of the tube.

9. Dissolve the solution with 50-150μL of reagent C to obtain a membrane protein sample.

10. The protein extract was quantified and divided into -80℃ refrigerator for reserve or directly used for downstream experiment.

Analysis of common problems:

1. Low protein concentration?

The abundance of membrane protein is relatively low, so it is necessary to increase the loading amount of cells as much as possible to increase the membrane protein concentration if conditions permit.

Some tissue samples may not be fully lysed when treated, resulting in low protein concentrations. Just extend the processing time of reagent A appropriately. It is best to handle under the condition of continuous oscillation, and it can be mixed with a suction head at intervals of several minutes without an oscillator.

2. What method is used to quantify the protein?

The BCA method is recommended. The Bradford method is not suitable because reagent A contains components that interfere with the Bradford method, resulting in inaccurate quantification. If dialysis has been performed or the buffer system has been replaced with a desalting column, the Bradford method can be used for quantification.

3. Is the extracted protein active?

This kit does not contain ionic detergent components, does not destroy the structure of the protein, does not destroy the original interaction between the proteins, and the proteins maintain their natural conformation and activity.

4. No bands in membrane protein electrophoresis?

Membrane protein samples are usually low in concentration, and protein quantification must be performed before electrophoresis to ensure that the amount of protein on the electrophoresis is sufficient. After the membrane protein is extracted and fully dissolved with the solution, it can be treated by ultrasound and then quantified.

After Loading the protein with Loading buffer, it can be kept at 50℃ for 30 minutes without boiling.

The final concentration of SDS in protein Loading buffer can be increased to 3%-10%.

If the content of membrane protein in some samples is too low, acetone can be used to precipitate the membrane protein, and then dissolve the membrane protein in the loading buffer, usually clear protein bands can be produced.

Low current and low current electrophoresis is the best method for electrophoresis.

Precautions:

1. This kit is intended for scientific research only and is not intended for diagnosis or treatment.
2. It is best to use disposable suction heads, tubes, bottles, or glassware, and reusable glassware

must be washed and thoroughly removed of residual cleaners before use.

3. All samples and exposed glassware should be disposed of in accordance with the prescribed procedure after the experiment is completed.
4. Avoid skin or mucous membrane contact with the reagent.
5. If the reagent accidentally comes into contact with skin or eyes, it should be rinsed with water immediately.